

COMPARISON OF THE REMOVAL OF RADIOSTRONTIUM FROM IN VIVO- AND IN VITRO-LABELED MILK BY ION EXCHANGE RESINS

L. F. EDMONDSON, D. H. KEEFER,¹ F. W. DOUGLAS, JR., J. Y. HARRIS,¹ AND
E. DODSON

Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA
Washington, D. C.

SUMMARY

Studies were made to determine differences in the amount of radiostrontium removed by ion exchange resins from milk labeled in vivo and in vitro with Sr^{85} or Sr^{90} , or both. The distribution of Sr^{85} and calcium between phases of centrifugally fractionated milk was also studied. The milks were adjusted to pH levels as low as 5.2 with citric acid before passing through ion exchange columns, and before centrifuging.

The amount of strontium removed varied from about 95% at pH 5.25 to 45% at pH 6.65 for in vitro-labeled milk. The amounts for in vitro-labeled milk were only slightly higher at low pH, but ranged from 79 to 50% at pH 6.65, for equilibration times of 30 min and 120 hr, respectively, before resin contact.

Portions of milk labeled in vivo with Sr^{85} were centrifuged at $105,000 \times G$ for 90 min. The amounts of strontium in the serum were 86, 46, and 19%, respectively, for pH levels of 5.2, 5.8, and 6.6. Corresponding calcium levels were 84, 59, and 33%. Practically all of the remainder was in the sediment. Less than 1% was recovered in the fat layer.

The removal of radiostrontium from milk by sulfonated polyethylene divinyl benzene ion exchange resins has been the subject of several investigations during the past few years (1-8). These resins were used in the single ion form such as Na^+ and Ca^{++} by some investigators (1-3, 7). More recently, they have been used in a mixed ion form including Ca^{++} , K^+ , and Na^+ , which is referred to as a CaKNa resin (6), or a mixed form including the above three cations plus magnesium (8). Regeneration of the resin with the appropriate ratio of these major milk cations results in a minimum of change in the cationic composition of milk treated with the resin. A further recent development has been the use of citric acid to adjust the pH of milk to 5.2 to 5.4 before contacting with the resin (4, 8). This results in the removal of over 90% of the radiostrontium.

The objective of the research in this laboratory has been to utilize synthetic resins to develop a feasible pilot plant process for removing radionuclides, particularly Sr^{90} , from milk.

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¹ Public Health Service, Department of Health, Education and Welfare, Washington, D. C.

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The development of a process necessarily includes a study of many factors affecting the removal, procedures for sanitizing and regenerating the resin, and of the effect of the process on the quality of the treated product. To study these factors, it is advantageous to use Sr^{85} rather than Sr^{90} . The latter is tedious and time-consuming to determine analytically. Also, environmental levels are too low to obtain the accuracy desired for research and development purposes. Sr^{85} is a gamma emitter and can be assayed rapidly without sample preparation. The isotopes of strontium, including Sr^{90} , Sr^{85} , Sr^{86} , and stable strontium, react the same chemically. The half-lives of Sr^{90} , Sr^{85} , and Sr^{86} are 28 yr, 64 days, and 51 days, respectively.

For the current studies Sr^{85} was either administered to a Holstein cow orally or intravenously, or added directly to milk in measured quantities. In most cases, levels of about $1 \mu\text{c}$ per liter of milk were used for the removal studies. Because of the convenience and economy of using in vitro labeled milk it is preferable to use this method, particularly when large quantities are needed. However, it is necessary to know the effects of the method of incorporating the strontium on its ease of removal by ion exchange and on its chemical and physical state in milk, such as degree of ionization and

complexing with other milk constituents. Its association with other milk constituents is reflected in its distribution between serum and sediment fractions of centrifugally separated samples. This technique and ion exchange removal methods were used to study the relationship between *in vivo* and *in vitro* methods of labeling milk with radiostrontium. The studies were made on milks at various pH levels.

EXPERIMENTAL PROCEDURE

Preparation of milks. Whole cow's milk *in vitro* labeled with Sr^{85} was obtained by adding approximately 1 μc per liter of milk and holding in a refrigerator for periods up to 120 hr. The lots of milk were stirred twice daily during the holding time. Milks *in vivo* labeled with Sr^{85} and Sr^{90} were obtained from a Holstein cow dosed orally or intravenously, so that the activity in the milk ranged from 0.5 to 1.0 μc per liter of milk. Specific activity of Sr^{85} was $> 500 \text{ mc/g}$; purity $> 98\%$ exclusive of $< 1\%$ Sr^{90} . Sr^{90} was used in one experiment so that oral and intravenous methods of labeling the milk could be compared at the same time. In the other trials Sr^{85} was used. Oral doses required for this level of activity were about 1 mc per day. Intravenous injections required about 0.1 mc per dose.

The pH of the milk was varied by adding, with thorough stirring, a solution of 0.75 M citric acid until the desired pH was obtained. In trials where the pH was returned to normal after acidification, 0.75 M KOH was used.

Resin regeneration and column operating procedure. Commercial grade Amberlite IR-120 (Na^+) ion exchange resin, 16-50 mesh size, was regenerated in columns by passing a mixed solution of the chlorides of calcium, magnesium, potassium, and sodium through until the resin was equilibrated with the solution. The composition of the regenerating solution, in grams per liter, was as follows: $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 44.5; KCl, 29.8; $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$, 10.2; and NaCl, 14.2. Ideally, when it is desired to maintain the milk cationic composition, the resin regenerate composition varies with the pH of the milk to be treated (5). For example, resins used to treat milks at low pH should contain a larger mole fraction of calcium. However, the differences in the desired resin composition for the pH values studied do not significantly affect the amount of strontium removed. Consequently, the above solution was used for all experiments in this series, since the amount of strontium removed was the only analytical result of interest.

After regeneration, excess solution was washed from the resin with distilled water. The water was then drained to the top of the resin bed before passing milk through it.

The columns were 21 mm in diameter and filled to a depth of 30.5 cm with back-washed and settled resin (105 ml of resin bed). The milk was passed downflow at a rate of 0.25 resin bed volumes (rbv) per minute. The temperature was maintained at $14 \pm 2^\circ \text{C}$.

Centrifugal fractionation of milks. Portions of the *in vivo*-labeled milk were adjusted to various pH levels and centrifuged at $105,000 \times G$ for 90 min at a temperature of 60°F in a Model L Spinco centrifuge.³ The serum, sediment, and fat layers were separately recovered and analyzed for radiostrontium and calcium. Under these conditions the sediment occurs as a firm semi-solid, adhering to the bottom of the centrifuge tube.

The results tabulated, and those used for construction of the figures, are the average of two separate trials.

Determination of radiostrontium. The Sr^{85} (gamma) was determined by gamma spectroscopy using a 3-in. NaI (Tl = activated) well-crystal, single-channel analyzer system. Sr^{90} was counted in a gas flow proportional counter connected to a scaler system. Interference from Sr^{85} was corrected for by using the observed counting efficiency of Sr^{90} in the proportional counter and the Sr^{85} activity in each sample as determined in the single-channel gamma spectrometer.

RESULTS

Table 1 shows the amount of radiostrontium removed by the ion exchange resin from milk

TABLE 1

Per cent radiostrontium removed by ion exchange resin from *in vivo*-labeled milk obtained by oral and by intravenous administration

| pH of milk | 5.4 | 5.8 | 6.2 | 6.65 |
|---------------------|-----------------|------|------|------|
| | — (% removed) — | | | |
| Oral administration | 90.5 | 69.0 | 52.3 | 44.5 |
| Iv administration | 91.5 | 67.5 | 51.3 | 42.8 |

labeled *in vivo* by oral and by intravenous administrations. Sr^{85} was given orally and Sr^{90} by iv injection to the same cow at the same time. Simultaneous administration affords a means of minimizing experimental errors inherent in working with different lots of milk.

³ Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

The data show no significant differences between the two procedures of dosing the cow.

The removals of Sr^{85} from in vivo- and in vitro-labeled milks by ion exchange resin at pH 5.25 and 6.65 (normal milk) are compared in Figure 1. In vitro-labeled milks in these

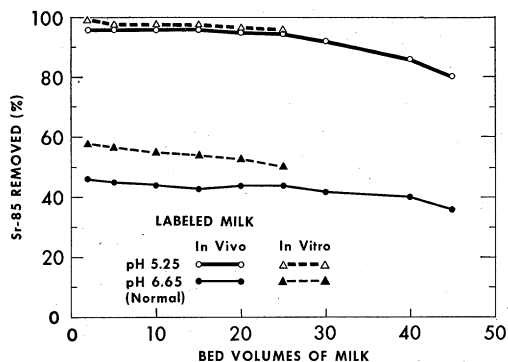


FIG. 1. The removal of Sr^{85} from in vivo- and in vitro-labeled milk by ion exchange resin (flow rate = 0.23 ml/min/ml of resin; in vitro-labeled milk was equilibrated for 72 hr before resin treatment).

trials were held in a refrigerator (equilibrated) for 72 hr before passing through the resin. The amount removed is plotted as a function of the volume passed through. In Figure 2 the

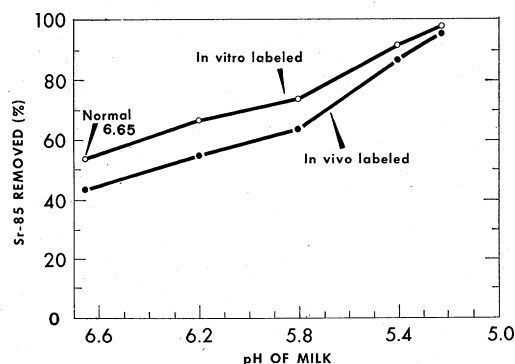


FIG. 2. Comparison of the removal of Sr^{85} from cow's milk at various pH levels when labeled in vivo and in vitro with the isotope.

average per cent removal for the first 20 resin bed volumes are compared for different pH values. At lower pH levels the difference between the two procedures of labeling becomes less. At pH 5.25 the difference is very small. The results show good agreement with similar studies made by Murthy et al. on goats' milk (8).

In Table 2 the effect of equilibration time on the amount of strontium removed is shown at

TABLE 2

Per cent removal of Sr^{85} from in vitro-labeled milk equilibrated for various times, and from in vivo-labeled milk by ion exchange resin (Average of first 20 bed volumes through columns)

| pH of milk | In vitro-labeled milk Equilibration time (hr) | | | | | In vivo-labeled |
|------------|--|----|------|------|------|-----------------|
| | 0.5 | 4 | 24 | 72 | 120 | |
| | (% removed) | | | | | |
| 5.4 | 98 | 97 | 95.2 | 92.2 | 91.5 | 88.3 |
| 6.65 | 79 | 70 | 60.7 | 54.7 | 49.7 | 45.0 |

pH 5.4 and 6.65. Also included are data for in vivo-labeled milks. The data show that the longer the in vitro-labeled milk is equilibrated the less the amount of strontium removed by ion exchange. However, even after 120 hr of equilibration time, from 3 to 5% more was removed than from in vivo-labeled milk. The difference between methods of labeling milk was greatest when in vitro-labeled normal milk (pH 6.65) was passed through the resin within one-half hour after labeling.

Separation of colloidal components of milk by high-speed centrifugation affords a means of studying the degree of binding of alkaline earths by casein. For these studies, samples of centrifugally fractionated milks were analyzed as described under Procedures. The percentages of Sr^{85} and calcium in the serums are shown in Figure 3 as a function of pH. At all pH levels practically all of the remainder was in the sediment. Less than 1% was recovered in the fat phase. There was considerably more calcium than Sr^{85} in the serum at normal pH. About 33% of the total calcium and 19% of the strontium remained in the serum. The difference decreased with decreasing pH. About 85% of each was recovered in the serum at

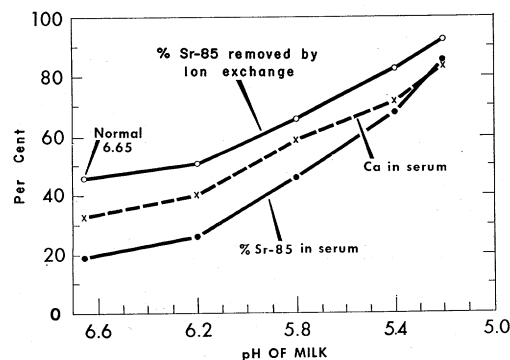


FIG. 3. Effect of pH on the amount of Ca and Sr^{85} in the serums of milk and on the amount of Sr^{85} removed by ion exchange resin. (Milk was in vivo-labeled with Sr^{85} .)

pH 5.2. Twardock et al. (9) used high-speed centrifugation to study the distribution of Ca^{45} and Sr^{85} between serum and sediment fractions of normal goats' milk labeled in vitro with the isotopes. The Ca^{45} content in the serum decreased from 44% of the total at 20 min after adding the isotope to 34% at 150 hr after the addition. A similar decrease in the serum phase with time was also noted for Sr^{85} . The per cent of the radionuclides in the serum of goats' milk labeled in vivo was 28 and 19%, respectively, for Ca and Sr. As with the ion exchange studies, this also shows that these cations, when added in vitro, equilibrate very slowly with other milk constituents.

Also plotted in Figure 3 are the amounts of Sr^{85} removed by ion exchange. At pH 6.65 (normal milk) about 47% was removed by ion exchange (average of first 20 resin bed volumes); whereas, only 19% occurred in the serum phase. Assuming all of the serum strontium to be removed by ion exchange, it may be calculated that about one-third of the colloidal strontium was also removed $\left(\frac{47\% - 19\%}{100\% - 19\%} \right)$.

Studies on the effect of readjustment of the pH of milk before passing through the resin columns on Sr^{85} removal were made by acidifying to pH 5.2. (The milk was in vivo-labeled with Sr^{85} .) The milk was then divided into three lots readjusted to pH levels of 5.8, 6.2, and 6.65, respectively, with 0.75 M KOH. These lots were further divided into sublots passed through ion exchange columns at various time intervals after neutralizing. Fifteen resin bed volumes of effluent milk were collected, from which samples were taken for Sr^{85} assay.

The results are given in Table 3. The data show that there is little reduction in the amount removed when the pH was readjusted to 5.8 and 6.2, then treated with the resin after 20

min when compared to treatment at pH 5.2. Readjustment to normal pH, however, shows about 12% less removal. Treatment with the resin 4 hr after readjustment to pH 5.8 and 6.6 resulted in removal of 92.9 and 81.8%, respectively. The removal tended to level off at near these values up to time intervals of 48 hr. Included in the table for the 20-min interval are data in parentheses, showing the amounts removed when the pH was lowered only to the value listed. No readjustment was involved.

DISCUSSION

The alkaline earths, including calcium and strontium, form complexes with a great many substances. The role of calcium in complex formation with phosphates and caseinate in milk has been studied extensively, because it comprises a large proportion of milk ash, and because of its influence on milk properties. Strontium is insignificant in both respects. Accurate data on its content (stable form) in milk are unavailable because of its very low content—about 1.0 mg/liter. Efforts to remove the radioisotopes, Sr^{90} and Sr^{85} , quite naturally stimulate interest in the state of strontium in milk. The stability of its complexes and its reactivity in milk should parallel those of calcium.

The extent of removal (percentage wise) of environmental Sr^{90} (e.g., 10 μc /liter), and of tracer levels of Sr^{85} (1 μc /liter) will be the same as long as the method of incorporating them into milk is the same, since both levels are many orders of magnitude less than the stable (natural) strontium content. Ten microcuries of $\text{Sr}^{90} \approx 10^{-9}$ mg and 1 μc of $\text{Sr}^{85} \approx 5 \times 10^{-6}$ mg.

Figures 1 and 2 show two effects with respect to the binding of Sr^{85} in milk. The ease of removal at low pH is a measure of the shift from complexed strontium to ionized, or dissolved, strontium. This is a well-known influence of H^+ on stability of alkaline earth complexes. The fact that less is removed at a given pH from milks in vivo-labeled than from in vitro-labeled milks shows that metabolized strontium is complexed to a greater degree than the strontium added to the milk. The milk was refrigerated for 72 hr after in vitro-labeling in these studies. The difference between the amounts removed from milks labeled by the two methods is greatest at pH 6.65 (normal milk). This is a logical result of the shift in position of equilibrium between dissolved and colloidal strontium with changes in H^+ concentration.

The data in Table 2 show that considerable time is required for dissolved strontium (in

TABLE 3
Effect of readjustment of pH of milks acidified to pH 5.2 on removal of Strontium⁸⁵ by ion exchange resin

| pH | 5.2 | 5.8 | 6.2 | 6.65 |
|-------------------------------|-----------------|---------------------------|---------------------------|---------------------------|
| Time after readjustment of pH | — (% removed) — | | | |
| 20 min | 98.5 | 96.9 (64) ^a | 96.0 (52) ^a | 85.7 (43) ^a |
| 4 hr | | 92.2 | | 81.8 |
| 24 hr | | 93.5 | | 80.5 |
| 48 hr | | 93.8 | | 80.4 |

^a Removal when pH was lowered to the value listed. Milk was passed through resin column. No readjustment was involved.

vitro added) to bind with the colloidal or other milk constituents. In milk, the equilibrium between dissolved and complexed forms is a precarious one and the shift from one form to another is probably not a simple exchange rate. Most of the strontium is bound to the caseinate-phosphate complex. This complex exists as aggregates of micelles with diameters up to 300 m μ . Consequently, strontium (or calcium) bound within the micelles is expected to be restricted by considerable physical hindrance. Dispersal of the caseinate micelle must precede the change from dissolved to bound form, to attain the metabolized state. Apparently, this is a very slow change.

The fact that over twice as much strontium was readily removed by ion exchange (Figure 3) at pH 6.65 as was obtained in the serum shows that a considerable portion of the bound strontium was readily available for exchange. However, over 50% of it was not removed at this pH. This again is a reflection of the extent of binding within the large caseinate micelles.

The experiment on readjustment of the pH of acidified milk (Table 3) shows that the original caseinate complex is not obtained simply by readjusting the pH. It is probable that the original state of micelle dispersion would never be completely reversed. Although the removal of strontium, at given pH levels, from acidified (pH 5.2)-neutralized milk was much greater than from milk having been acidified only to the given level, for practical use there is no advantage in neutralizing before the resin treatment.

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